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Metal Carbonyls for site-specific FTIR Microscopic Localization

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Introduction: FTIR microspectroscopy has been used to demonstrate differences between diseased and normal tissue, changes in the chemical composition that are dependent on the state of differentiation, and metabolic differences between cellular layers [1]. Recent developments in infrared array detectors (FCA or focal plane array detectors) have led to the collection of spectroscopic images with unprecedented fidelity [2]. Metal carbonyl complexes have been successfully conjugated to proteins and nucleic acids [3,4] and their intense infrared absorption bands in the region 2200 to 1850 cm^{-1} have led to their use as non-isotopic organometallic markers for immunoassays [5]. We wish to develop metal carbonyls as site-specific probes or “IR tags” for FTIR Microscopy.

Methods and Materials: Metal carbonyls, Teflon, nylon, cellulose membranes.

Results: Phosphine derivatized mono-tungsten, tri-osmium, and tetra-iridium carbonyls (100 E-9 M to 25 E-12 M) were spotted and dried on PTFE (Teflon), nitrocellulose, and nylon membranes, and their FT-IR spectra were recorded. As low as 100 E-12 M of the mono-phosphine substituted tri-osmium and the tetra-iridium carbonyls and 250 E-12 M of the tetra-phosphine substituted tetra-iridium carbonyl could be detected. Both Globar and Synchrotron sources gave similar detection limits when 50X50 µM aperture was used. Signal to noise ratios was significantly higher using 20X20 µM aperture for the Globar Source as expected. The signal went down by 80% when the aperture was reduced from 50X50 µM s to 20X20 µM and remained the same at 10X10 µM for the Synchrotron source. This can be attributed to the sample area covered by the aperture; the sample spots were between 3X3 and 4X4 mM with nearly uniform concentration. This suggests that higher detection limits could be obtained by reducing the size of the sample spot.

Twenty-five micrograms of the spotted target antibody could be detected with mono-phosphine substituted tetra-iridium carbonyl labeled secondary antibody. This can be attributed to two binding sites on the target antibody and multiple metal carbonyl labels (4-6) per secondary antibody molecule. Since 10 E-12 g of the antibody can be detected by other methods, e.g., colloidal gold labeled antibody and silver metallography, further Dot-blot assays will be carried out using biotinylated secondary antibody followed by the labeled-streptavidin, -streptavidin-albumin, and -streptavidin-thyroglobulin conjugates.

Conclusions: Sensitivities of detection of metal carbonyls depend upon the number of bound carbonyl ligands to the metal. The detection limits obtained with FTIR are much lower than conventional method, such as gold-silver autometallography. Reagents labeled with multiple metal carbonyls will be tested in future to improve their sensitivity of detection using FTIR microspectroscopy.

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